was precipitated. The resuspended precipitate was analyzed by SDS-PAGE and stained with Coomassie brilliant blue.

Figure 10 depicts a photograph of an SDS-PAGE gel. VEGF-2 was purified from the medium supernatant and analyzed by SDS-PAGE in the presence or absence of the reducing agent b-mercaptoethanol and stained by Coomassie brilliant blue.

Figure 11 depicts reverse phase HPLC analysis of purified VEGF-2 using a RP-300 column (0.21 x 3 cm, Applied Biosystems, Inc.). The column was equilibrated with 0.1% trifluoroacetic acid (Solvent A) and the proteins eluted with a 7.5 min gradient from 0 to 60% Solvent B, composed of acetonitrile containing 0.07% TFA. The protein elution was monitored by absorbance at 215 nm ("red" line) and 280 nm ("blue" line). The percentage of Solvent B is shown by the "green" line.

Figure 12 shows a schematic representation of the pHE4-5 expression vector (SEQ ID NO:9) and the subcloned VEGF-2 cDNA coding sequence. The locations of the kanamycin resistance marker gene, the VEGF-2 coding sequence, the oriC sequence, and the *lac*Iq coding sequence are indicated.

Figure 13 shows the nucleotide sequence of the regulatory elements of the pHE promoter (SEQ ID NO:10). The two *lac* operator sequences, the Shine-Delgarno sequence (S/D), and the terminal *Hind*III and *Nde*I restriction sites (italicized) are indicated.

Figure 14A-D shows that VEGF-2 treatment increases the level of rhodopsin protein and the number of photoreceptor cells. Dissociated retinal cells were prepared from P1 animals, plated at a density of 425 cells/mm^2 and treated with VEGF-2 (A and B) or VEGF-2 (C and D). After 2 (open squares), 5 (solid squares), 7 (open circles), or 9 (solid squares) days, the total number of cells in the cultures was estimated by measuring the calcein emission. The cultures were then fixed and the levels of rhodopsin protein quantitated by ELISA.

Figure 15 shows that the number of rhodopsin immunopositive cells increased as a function of VEGF-2 concentration. The retinal cells were maintained in vitro for 8 days in the presence of either VEGF-1 or VEGF-2. The cultures were then fixed and immunohistochemically stained for rhodopsin.

Figure 16A-C shows that VEGF-2 increases BrdU and [3H] thymidine incorporation in retinal cultures in a developmentally restricted manner. The cells were isolated from P1 animals and plated at a density of 425 cells/mm^2.

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The cultures were initially treated 4 hours after plating with either VEGF or VEGF-2. After 1, 2, or 3 days, the cultures were labeled for 4 hours with BrdU. The cells were then fixed and processed for BrdU immunohistochemistry.

Figure 17A-B shows the loss of the response to VEGF-2 or VEGF-1 as a function of the time lapsed between the isolation of the cells and the initial addition of the factors. One set of cultures was initially treated with factors 4 hours after plating (9/0) and subsequently, additional sets were treated after 24 or 48 hours (8/1 or 7/2, respectively). After 9 days in culture, the cells were fixed and the level of rhodopsin protein was quantitated by ELISA assay.

Figure 18A-C shows VEGF increases the number of Amacrine but not Muller or Endothelial cells. Retinal cells were treated for 8 days with the indicated concentrations of VEGF-2. The cells were then fixed and immunohistochemically stained for syntaxin (A), analyzed for the level of high-affinity GABA uptake (B), or GFAP (C).

Figure 19A-C shows the effect of developmental age on the response to VEGF-2. Retinal cells derived from E15 (A), E20 (B) or P1 (C) animals were plated at a density of 212 (open squares), 318 (solid squares), or 425 cells/mm^2. Four hours after plating, the cultures were treated with the indicated concentrations of VEGF-2. After 24 hours, the cultures were switched to serum-free medium and the factors were added again. The cultures were then labeled with [3H] thymidine after 48 hours.

Figure 20A-B compares the response of retinal cells to VEGF-2 and other factors. The cultures were seeded at a density of 425 cells/mm^2 and treated for 9 days. Panel A shows the total number of cells in the cultures was estimated using calcein, while panel B shows the level of rhodopsin protein determined by ELISA assay.

Figure 21A-C shows that CNTF inhibits the response of the photoreceptor cell progenitors to VEGF-2. Retinal cultures were treated 24 hours after plating with the indicated concentrations of CNTF in the presence or absence of 150 ng/ml of VEGF-2. After 8 days in vitro, the amount of rhodopsin protein was quantitated (A) and the total number of cells in the cultures was determined (B). (C) To determine the effect of CNTF treatment on the early proliferative response induced by VEGF-1, the cultures were treated with the indicated concentrations of VEGF-2 in the presence or absence of 100 ng/ml CNTF. After 48 hours, the cultures were labeled for 4 hours with [3H] thymidine.

Figure 22 shows the enhanced LEC proliferation in response to VEGF-2 and antibody treatment.

Figure 23 shows LEC proliferation in response to VEGF-2 and VEGF-2:antibody combination.

Figure 24 shows the epitope map for murine anti VEGF-2 monoclonal antibodies.

Figure 25 shows the status of the murine VEGF-2 monoclonal antibodies.

Detailed Description of the Preferred Embodiments

In accordance with one aspect of the present invention, there are provided isolated nucleic acid molecules comprising a polynucleotide encoding a VEGF-2 polypeptide having the deduced amino acid sequence of Figure 1 (SEQ ID NO:2), which was determined by sequencing a cloned cDNA. The nucleotide sequence shown in SEQ ID NO:1 was obtained by sequencing a cDNA clone, which was deposited on May 12, 1995 at the American Type Tissue Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, and given ATCC Deposit No. 97149.

In accordance with another aspect of the present invention, there are provided isolated nucleic acid molecules comprising a polynucleotide encoding a truncated VEGF-2 polypeptide having the deduced amino acid sequence of Figure 2 (SEQ ID NO:4), which was determined by sequencing a cloned cDNA. The nucleotide sequence shown in SEQ ID NO:3 was obtained by sequencing a cDNA clone, which was deposited on March 4, 1994 at the American Type Tissue Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, and given ATCC Deposit Number 75698.

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373 from Applied Biosystems, Inc.), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least